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ORIGINAL PAPER

Genomic associations for drought tolerance on the short arm of wheat chromosome 4B

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Abstract Drought is a major constraint to maintaining yield stability of wheat in rain fed and limited irrigation agro-ecosystems. Genetic improvement for drought tolerance in wheat has been difficult due to quantitative nature of the trait involving multiple genes with variable effects and lack of effective selection strategies employing molecular markers. Here, a framework molecular linkage map was constructed using 173 DNA markers randomly distributed over the 21 wheat chromosomes. Grain yield and other drought-responsive shoot and root traits were phenotyped for 2 years under drought stress and well-watered conditions on a mapping population of recombinant inbred lines (RILs) derived from a cross between drought-sensitive semidwarf variety "WL711" and drought-tolerant traditional variety "C306". Thirty-seven genomics region were identified for 10 drought-related traits at 18 different chromosomal locations but most of these showed small inconsistent effects. A consistent genomic region associated with drought susceptibility index (*qDSI.4B.1*) was mapped on the short arm of chromosome 4B, which also controlled grain yield per plant, harvest index, and root biomass under drought. Transcriptome profiling of the parents and two RIL bulks with extreme phenotypes revealed five genes underlying this genomic region that were differentially expressed between the parents as well as the two RIL bulks, suggesting that they are likely candidates for drought tolerance. Syntenic genomic regions of barley, rice, sorghum, and maize genomes were identified that also harbor genes for drought tolerance. Markers tightly linked to this genomic region in combination with other important regions on group 7 chromosomes may be used in marker-assisted breeding for drought tolerance in wheat.

Keywords Drought tolerance · Expression profiling · Genomic associations · Root traits · Wheat

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Introduction

Adequate soil moisture is essential for proper growth and development of crop plants which ultimately leads to optimum productivity. But today, drought is seen as one of the major environmental constraints restricting crop productivity. Unfortunately, the underlying causes for this are unavoidable that include global warming, depletion of underground water table, and erratic rainfall patterns leading to scarcity of water in agro-ecosystems worldwide, particularly in the semiarid, subtropical, and tropical dry lands (Baltas et al. 2010). One of the most promising and economically viable solutions for increasing yield stability of crops in these regions is through genetic improvement by

introgression of genes and genomic regions for drought tolerance into high-yielding cultivars (Blum 1988; Nevo and Chen 2010).

Wheat has a large genome of about 17,000 Mb and drought tolerance is a complex trait comprising of a number of physiobiochemical processes at the cellular and organism levels at different stages of the plant development. Hence, it has lagged behind in development of drought-tolerant varieties using only conventional breeding approaches. Plants adapt to drought stress in various ways, including enhanced water uptake by developing large and deep root systems, reduced water loss by increasing stomatal resistance, and adaptation to water shortage by accumulation of cellular osmolytes (Rampino et al. 2006). Development of high-density molecular linkage map provides a tool for dissecting the genetic basis of such complex traits into component quantitative trait loci (QTLs) through genomic associations. Although substantial amount of information has been generated on the genetics of above ground traits under water stress, limited attention has been paid to below the ground root traits in wheat. Studies in rice have shown that strong root growth is an important factor for drought tolerance (Yoshida and Hasegawa 1982; Ray et al. 1996). Root thickness, dry weight, volume, and density are high heritability traits that show positive association with drought tolerance in rice (Ekanayake et al. 1985; Qu et al. 2008). Breeding for an efficient root system is important for improving rice productivity in rainfed environments (Yadav et al. 1997; Price et al. 2002; Li et al. 2005). However, root traits are difficult to evaluate since removing intact roots from soil is tedious and root morphological characteristics are easily influenced by the environment. Most of the reported QTLs for drought tolerance in wheat are for yield and yield components under water-limited conditions (Quarrie et al. 2006; Kirigwi et al. 2007; Maccaferri et al. 2008; Mathews et al. 2008; McIntyre et al. 2010). Limited information is available on QTLs for root traits in wheat, e.g., Ma et al. (2005) mapped a QTL for root growth rate under aluminum treatment. QTLs for number and length of primary/lateral roots and root dry matter under control and nitrogen-deficient conditions have also been identified in wheat (Laperche et al. 2006). Relative root growth has been used as a parameter to map QTLs for tolerance to boron toxicity in barley (Jefferies et al. 1999). A dramatic reduction in grain yield occurs when drought coincides with the irreversible reproductive processes, making the genetic analysis of reproductive stage drought tolerance crucially important (Cruz and O'Toole 1984; Price and Courtois 1999; Boonjung and Fukai 2000; Pantuwan et al. 2002). However, QTLs for root traits under reproductive stage drought stress have not yet been reported in wheat.

In the present study, we evaluated the shoot and root traits of wheat under control and water stress conditions to identify the genomic regions associated with reproductive-stage drought tolerance. We particularly focused on identification of candidate genes for drought tolerance that were differentially expressed and also co-located in the identified genomic regions. Further, we tried to identify drought-responsive genes in the syntenic chromosomal regions of rice, sorghum, and maize.

Materials and methods

Plant material and drought stress treatment

A mapping population of 206 F_9/F_{10} recombinant inbred lines (RILs) used in this study was developed from a cross between high-yielding drought-susceptible wheat variety WL711 and a traditional low-yielding but drought-tolerant variety C306 (Aggarwal and Sinha 1987; Sharma and Kaur 2008). Single wheat plants were grown in 15×100 cm polyvinyl chloride (PVC) pipes in two successive years for phenotypic evaluation. Each pipe was loaded with thoroughly mixed soil composed of three parts soil from wheat field and one part vermicompost. The parental lines and RILs were grown under control and drought conditions in three replications in each of the 2 years. Three germinated seeds were sown directly in each pipe and only one healthy seedling was retained at 20 days after sowing. At the beginning of the tillering stage, 1 g of urea (dissolved in water) was applied to each pipe. The plants were fully irrigated by watering every day until the start of drought treatment. The supply of water was stopped at the booting stage in order to apply drought stress at flowering. Control plants were maintained by continued irrigation. Rain was kept out by covering the pipes with transparent polythene sheets during rainy days.

Phenotypic evaluation

Ten traits were evaluated including six for above-ground parts and four for below-the-ground traits of the wheat plant. The above-ground traits included grain yield (GY), shoot biomass (SB), plant height (PH), days to flowering (DTF), harvest index (HI), and drought susceptibility index (DSI) estimated according to Fischer and Maurer (1978) as follows: DSI=(1−GYds/GYns)/DII, where GYds and GYns are means of single plant yields of a given genotype in drought stress (DS) and nonstress (NS) environments, respectively. The DII for each trial was calculated as DII=1−Xds/Xns, where Xds and Xns are the means of all genotypes under DS and NS environments, respectively. Below-the-ground traits evaluated at seed maturity included maximum root length (MRL), total root biomass (TRB), root biomass up to 30 cm (RBU30), and root biomass below 30 cm

(RBB30). For the measurement of root traits, PVC pipes containing soil and roots were soaked in a water tank (3×1×1 m) for 12 h to loosen the soil. After this, pipes were laid down on a 2-mm sieve screen frame and force of water was applied slowly from both sides of the pipes to loosen and remove the soil and collect the intact roots. The roots were cut at 30 cm from the basal node of the plant to divide it in two parts, RBU30 and RBB30, representing shallow and deep roots, respectively (Fig. 1).

Linkage map construction and QTL analysis

Leaves from 1-month-old wheat plants were used for DNA extraction by CTAB method with minor modifications (Murray and Thompson 1980). Total 730 simple sequence repeats (SSR) markers developed by IPK Gatersleben (*gwm/gdm*), Wheat Microsatellite Consortium (*wmc*), Beltsville Agricultural Research Station (*barc*), and INRA collections (*cfd/cfa*), as described at the GrainGenes website (www.wheat.pw.usda.gov) and 44 expressed sequence tag (EST)–sequence-tagged site (STS) markers (Singh et al. 2007) were tested for polymorphism between two parents. The STS markers monomorphic between parents were analyzed for cleaved amplified polymorphic sequence (CAPS) using restriction enzymes *RsaI*, *DpnI*, *HhaI*, *BsuRI*, *MseI*, *BamHI*, and SNP using denaturing high-performance liquid chromatography (DHPLC; Schwarz et al. 2003; Lai et al. 2005). PCR products of SSR markers were first separated by electrophoresis in 4 % metaphor agarose gel using 1× TBE buffer and those found monomorphic were separated in 8 % polyacrylamide gel for higher resolution. The CAPS markers were resolved in 2 % agarose gel due to larger fragment size differences. The polymorphic markers were analyzed in all the 206 RILs and molecular linkage map was constructed using Mapmaker/EXP 3.0 software (Lincoln et al. 1992). Markers were assigned to individual wheat chromosomes based on the microsatellite consensus map (Somers et al. 2004) and the composite wheat map (www.wheat.pw.usda.gov) by giving command, "group" with LOD score 3.0, and then "order" to develop the linkage map. Kosambi function was used to convert recombination frequencies into centi Morgan values (Kosambi 1944). Graphical representation of the linkage maps was made using MapChart version 2.2 (www.biometris.wur.nl). QTL analysis for each trait was carried out using mean values of the three replicates in each of the 2 years separately using QTLNetwork version 2.0 software based on a mixed linear model (Wang et al. 1999; Yang and Zhu 2005). Composite interval mapping was done using forward–backward stepwise, multiple linear regression with a probability into and out of the model of 0.05 and window size set at 10 cM. A QTL was declared significant if the phenotype was associated with a marker locus at P values of <0.05 after 1,000 permutations for selecting the F value threshold.

Fig. 1 Variation in root growth of wheat parental lines WL711 (left side to the ruler) and C306 (right side to the ruler) under control (left) and drought (right) conditions. A meter scale is included to show the actual size of the root systems

Transcriptome profiling

Ten of each extreme tolerant and susceptible RILs were identified on the basis of their DSI for grain yield and used for transcriptome profiling. Flag leaves were collected from control- and drought-stressed plants 5 days after anthesis and snap frozen in liquid nitrogen for RNA extraction because flag leaf is considered more important for grain yield than any other leaves (Aprile et al. 2009). Relative water content (RWC) was estimated as RWC=[(fresh weight−dry weight)/(turgid weight−dry weight)] at the time of leaf collection to check whether the plants were actually under water stress (Ergen et al. 2009). The RWC of C306 and WL711 in control conditions was 89 and 85 %, whereas it was reduced to 73 and 66 % under water stress, respectively. Total RNA was extracted from frozen leaves using TRIzol (Sigma) and processed according to Affymetrix Gene Chip Expression Analysis Technical Manual (Affymetrix, Inc., Santa Clara, CA, USA). Single- and double-stranded cDNAs were synthesized using Superscript Double-Stranded cDNA Synthesis Kit (Invitrogen Corp., Carlsbad, CA, USA). The resulting ds-cDNA was column purified and used as template to generate biotin-tagged cRNA using in vitro transcription reaction (IVT), from Affymetrix GeneChip IVT Labelling Kit. Biotin-labeled cRNA (15 μg) was then fragmented and hybridized to Affymetrix GeneChip® Wheat Genome Array (containing 61,127 gene probes) for 16 h at 45 °C at 60 rpm using Hybridization Wash and Stain Kit (Affymetrix) in Fluidics

Station 450 following the manufacturer's protocol and scanned using GeneChip® Scanner 3000 with GeneChip® Operating Software.

Microarray hybridization was carried out in biological triplicates (separate total RNA isolation and cRNA labeling for each hybridization) of control- and drought-stressed plants, making use of a total of 24 Affymetrix Gene Chip® wheat genome arrays. The array data were analyzed using Gene Chip Operating Software version GCOS 1.4 (www.affymetrix.com) and Gene Spring software version GX 11.0 (www.chem.agilent.com). We used a default target intensity value setting of $n=500$ and scaling factor of 3.1–8.5 for the array. The detection calls (present, absent, or marginal) for the probe sets were made by GCOS. Normalization was performed using the robust multichip average algorithm by Gene Spring and only gene expression levels with statistical significance ($P<0.01$) above the background levels were recorded as being "present". Genes with expression levels below this statistical threshold were considered as "absent." For differential expression, only those genes with $P\leq0.01$ and fold change >2.0 were considered to be up- or down-regulated. Microarray data from this study have been deposited at National Center for Biotechnology Information (NCBI)Gene Expression Omnibus with accession number GSE30436 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE30436). Annotation of the drought-regulated probes was done using NetAffyx software of Affymetrix and further validated using BLASTX search of NCBI.

Results

Phenotypic variation for drought-responsive traits in the parents and RILs

The two parents and 206 RILs were grown in PVC pipes in triplicates for the phenotyping of drought-responsive traits for 2 years. Transgressive segregation was observed in the RILs for each of the 10 traits investigated, indicating that alleles for higher phenotypic values were present in both the parents (Electronic supplementary material (ESM) Table 1). Yield was reduced under drought stress on an average by 57 % in the year 2009 and 69 % in 2010 as compare to the control conditions. This indicates that a relatively severe stress was imposed in 2010 as compared to 2009. The range of yield reduction in the RILs was 26–92 % in 2 years, suggesting that a moderate to severe level of reproductive stage drought stress was attained, which was adequate for the QTL mapping study. Drought-tolerant variety C306 showed higher yield stability than WL711 with a lower average percentage (63 %) of yield reduction under drought in the 2 years as compare to 75 % yield reduction in WL711. Further, C306 showed significantly lower DSI values of 0.88 and 0.90 as compare to 1.07 and 1.06 for WL711 in the year 2009 and 2010, respectively (ESM Table 1). Drought stress reduced harvest index, shoot biomass, and plant height as compared to control, indicating damage to growth and development of all plant organs under water stress that has a direct impact on yield. The RILs also behaved in a way similar to the individual parents in control and drought stress conditions, with expected segregating for the level of damage due to drought stress (ESM Table 1).

Standard statistical analyses were carried out using the Microsoft Office Excel 2007, SPSS_18, and MSTAT-C (Panwar et al. 2011). A combined analysis of variance (ANOVA) was performed over all environments. The combined ANOVA indicated statistically significant main effects for year, environment (control and drought), genotype, and genotype × environment (G × E) interactions for different drought-related traits, while variance due to replication was not significant (Table 1). The two parents were significantly different in their response to drought stress, even though there were distinctive genotypic divergences between them under control condition. The variations observed for each trait due to genotype, treatment and genotype × treatment interaction were statistically significant. The relative magnitudes of variance due to genotype and genotype × treatment interaction varied among traits. Variance due to genotype × environment interaction was substantially lower than variation due to genotype for all the traits except RBU30 where the magnitudes were almost equal, suggesting that the phenotypic variance for a particular trait was attributable to both genotype and the treatment effects.

Drought-tolerant variety C306 had a significantly higher root biomass than WL711 in both the years in control as well as stress conditions. Analysis of variance showed that between parent variation for root traits was significant in both the years ($P<0.001$). In drought conditions, the root length and root biomass were reduced as compared to control, indicating that below-ground traits were also affected by drought stress similar to the above-ground traits, except for RBB30 in C306 which was higher under drought condition as compared to control in both the years (ESM Table 1; Fig. 1). However, the difference in RBB30 between the two parents under drought was statistically significant only in 2009 with moderate drought condition.

Correlation among traits in the RIL population

Correlation coefficients among the 10 traits were investigated for drought and control conditions over 2 years (Tables 2 and 3). Under drought stress, grain yield per plant showed significant negative correlations with DSI and DTF in both the years and with TRB and RBB30 in 2010, when stress was relatively severe. Grain yield was highly positively

Table 1 Analysis of variance for drought-responsive traits of wheat plants grown in pipes under irrigated and drought environments for 2 years

Source of variation	*df*	GY	HI	SB	PH	DTF	MRL	TRB	RBU30	RBB30
Mean squares										
Replicates	2	3.66 ns	12.49 ns	12.13 ns	14.62 ns	0.83 ns	26.14 ns	20.19 ns	9.79 ns	17.19 ns
Genotypes	205	14.56**	107.303**	60.77**	423.04**	80.05**	177.01**	12.3**	3.56**	6.00**
Treatment	1	28,084.31**	32,272.58**	12,482.25**	45,505.25**	16,858.88**	9,329.55**	843.42**	193.69**	283.43**
Genotype x treatment	205	8.75**	95.46**	45.26**	85.06**	18.95**	122.88**	6.74**	3.01**	2.83**
Year	1	224.59**	3,914.56**	91,550.68**	83,190.03**	13.83**	22,895.42**	2,024.59**	269.62**	232.65**
Genotype x year	205	10.456**	54.70**	43.10**	103.89**	12.68**	58.63**	4.25**	2.58**	2.93**
Treatment x year	1	385.563**	4,394.10**	1,832.07**	7,017.82**	0.44**	660.69**	104.96**	6.12**	2.12**
G x treatment x trial	205	7.612**	62.77**	32.50**	60.48**	11.86**	64.77**	3.18**	2.70**	2.32**
Error	823	0.128	0.61	0.20	9.16	0.54	0.72	0.26	0.21	0.11
Genetic parameters										
CV		3.83 %	2.93 %	1.62 %	3.35 %	0.93 %	0.69 %	7.96 %	16.21 %	11.58 %
CD		0.47	1.09	0.63	4.21	1.03	1.19	0.714	0.636	0.456

GY grain yield per plant, *DSI* drought susceptibility index, *HI* harvest index, *SB* shoot biomass, *PH* plant height, *DTF* days to flowering, *MRL* maximum root length, *TRB* total root biomass, *RBU30* root biomass up to 30 cm, *RBB30* root biomass below 30 cm, *CV* coefficient of variation, *CD* critical difference, *ns* not significant

*P=0.05, significant correlation; **P=0.01, significant correlation

Table 2 Correlation coefficients among drought-related traits in 206 RILs derived from WL711/C306 under drought conditions in 2 years

Year	2009									2010								
Traits	GY	DSI	HI	SB	DTF	PH	MRL	TRB	RBU30	GY	DSI	HI	SB	DTF	PH	MRL	TRB	RBU30
DSI	−0.464**								−0.697**									
HI	0.695**	−0.476**								0.876**	−0.639**							
SB	0.212**	−0.035	0.468**							0.078	0.057	−0.204**						
DTF	−0.184*	0.114	0.006	−0.257**						−0.345**	0.266**	−0.336**	−0.054					
PH	0.236**	−0.032	0.189**	−0.016	−0.163*					−0.022	0.065	−0.078	0.256**	0.027				
MRL	0.111	−0.162*	−0.118	0.286**	−0.118	0.035				−0.079	0.042	0.010	0.000	0.010	−0.004			
TRB	0.046	−0.057	0.093	−0.136	0.265**	−0.018	0.113			−0.209**	0.239**	−0.236**	0.102	0.280**	0.152*	0.07		
RBU30	0.075	−0.073	0.151*	−0.094	0.203**	−0.018	0.099	0.639**		−0.006	0.034	−0.06	−0.13	0.206**	−0.126	0.127	0.594**	
RBB30	0.051	−0.056	−0.071	−0.028	0.194**	−0.008	0.104	0.734**	0.210**	0.152*	0.087	−0.262**	0.264**	0.230**	0.310**	0.021	0.687**	0.082

Abbreviations of traits are given in Table 1

*P=0.05, significant correlation; **P=0.01, significant correlation

Table 3 Correlation coefficients among drought-related traits in 206 RILs derived from WL711/C306 under control conditions in 2 years

Year	2009								2010							
Traits	GY	HI	SB	DTF	PH	MRL	TRB	RBU30	GY	HI	SB	DTF	PH	MRL	TRB	RBU30
HI	0.432**								0.775**							
SB	0.491**	−0.185**							0.364**	−0.242**						
DTF	−0.128	−0.185**	0.092						−0.102	−0.233**	0.150*					
PH	−0.071	−0.070	0.208**	0.108					0.041	−0.089	0.272**	0.087				
MRL	0.108	−0.011	0.133	0.143	−0.085				0.056	0.068	−0.064	0.075	−0.098			
TRB	0.136	−0.138	0.333**	0.258**	0.027	0.119			0.146	−0.013	0.234**	0.138	0.255**	0.017		
RBU30	0.203**	−0.007	0.191**	0.158*	−0.055	0.156*	0.577**		0.168*	0.090	0.120	0.091	0.030	0.02	0.654**	
RBB30	−0.023	−0.020	0.203**	0.143*	0.261**	−0.017	0.553**	0.231**	0.141	0.017	0.155*	0.123	0.351**	−0.052	0.702**	0.257**

Abbreviations of traits are given in Table 1

**P=0.01, significant correlation at probability level; *P=0.05, significant correlation at probability level

correlated with HI under drought stress in both the years but with SB and PH in 2009 and RBB30 in 2010 only. High positive correlation of HI with grain yield, its direct contribution to yield, and predominantly additive gene effects suggest that it could be used as a dependable trait for improvement of wheat productivity under drought. DSI showed highly negative correlation with yield and HI in both the years as expected, but it did not correlated with PH and SB in either of the 2 years, indicating that the plant height gene on chromosome 4B was not absolutely linked to the drought tolerance of C306 (Table 2). Under control conditions, grain yield was highly positively correlated with SB, HI, and RBU30. Lack of significant correlation with PH, DTF, MRL, TRB, and RBB30 suggests that these were not crucial for grain yield under control (Table 3). HI showed significant negative correlation with SB and DTF in both control and stress environments, except for DTF during 2009. Root biomass traits were significantly positively correlated with each other except for MRL which was not correlated (Table 2). Total root biomass was significantly correlated with RBU30 and RBB30 in both the years under both the environments. Under drought stress, TRB was significantly positively correlated with DTF. In 2010, when drought stress was relatively severe, root biomass was negatively correlated with GY and HI but positively correlated with DTF and plant height. MRL showed significant correlation with two traits under drought stress in 2009, a negative correlation with DSI and a positive correlation with shoot biomass. Under control conditions, MRL was not correlated with any of the traits, except for RBU30 in 2009 (Table 3). TRB was highly significantly correlated with the shoot biomass under control conditions in both the years. RBU30 was significantly correlated with yield per plant under control in both the years but not under drought stress. This suggested that RBU30 was not responsible for the drought tolerance in C306, but it was an important factor for higher yield of WL711 in well-watered conditions. In contrast, deep root biomass RBB30 was not correlated with yield in control condition in either of the 2 years, but was significantly correlated with yield under drought in 2010.

Construction of framework linkage map and QTL mapping

A total of 730 SSR and 44 STS loci were analyzed out of which 169 SSR (23.4 %) and one STS (2.27 %) showed polymorphism between the two parents and among RILs. The remaining 43 monomorphic STS loci were further analyzed for CAPS using different restriction enzymes and two of these showed CAPS polymorphism with restriction enzymes *BsuRI* and used for the genotyping of RIL population (Fig. 2). One more polymorphic locus was identified by further analysis for SNP polymorphism using DHPLC.

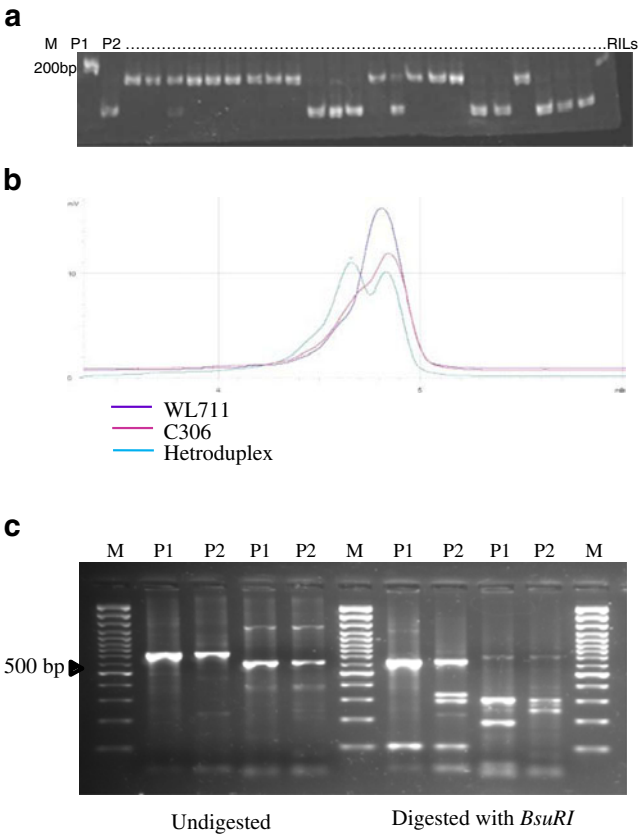


Fig. 2 Three different methods used for the genotyping of RILs: **a** parental lines and RILs genotyped using SSR marker "gwm577" by polyacrylamide gel electrophoresis, **b** SNP polymorphism between parents revealed by DHPLC, **c** SNP-CAPS polymorphism revealed by restriction enzyme *BsuRI*; *M* DNA size markers, *P1* WL711, *P2* C306

Thus, a total of 173 polymorphic loci, including 169 SSR, one STS, two CAPS, and one DHPLC marker were used for the genotyping of 206 RILs. The framework linkage maps were constructed using Mapmaker software (Fig. 3). The linkage maps of chromosome 2D and 3D showed the highest number of 12 markers each with a total map distance of 201 and 273 cM, respectively. Chromosome 5B and 6B showed the lowest number of five markers each covering map distance of 91.2 and 83.3 cM, respectively. Maps of all the 21 wheat chromosomes covered a total map distance of 3,720.2 cM with an average interval of 21.2 cM between the adjacent markers which was suitable for QTL analysis (Darvasi et al. 1993).

Genomic associations with grain yield and shoot traits

Genomic regions (QTLs) were identified for above-ground traits, including GY, HI, SB, PH, and DTF under drought stress and control conditions, as well as DSI for grain yield (Table 4). Five QTLs were identified for grain yield per plant in the 2 years; three of these were mapped on chromosome 2D, 3D, and 4B in 2009. Total phenotypic variation explained by these QTLs was 5, 13, and 6 %, respectively, with positive allele on 2D coming from WL711 and on 3D and 4B coming from C306. Similarly, three QTLs were identified on chromosomes 2D, 4B, and 5A in 2010, explaining phenotypic variation of 16, 7, and 6 %, respectively, with positive allele contributions on 2D and 5A from WL711, and 4B from C306. The only consistent QTL for grain yield under drought was located on chromosome 4B between marker interval barc20-gwm368 with positive allele coming from C306. A consistent QTL for drought susceptibility index (*qDSI.4B.1*) was also identified in the same marker interval on chromosome 4B. Total phenotypic variation explained was 14 and 7 % in the years 2009 and 2010, respectively, and the susceptibility allele was contributed by drought-sensitive parent WL711. The *qDSI4B.1* was co-located with QTLs for PH, SB, GY, and HI under stress; hence, it appears to be the most important QTL for drought tolerance in wheat variety C306 (Table 4; ESM Table 2). For HI under drought, one QTL (*qHI.4B.1*) was identified on chromosome 4B in 2009 and two QTLs (*qHI.2D.1*, *qHI.5A.1*) were identified on chromosomes 2D and 5A in 2010. The *qHI.5A.1* was co-located with a QTL for yield per plant in the year 2010. The HI QTLs were not consistent over the 2 years of testing, which could be partly due to a higher severity of drought in 2010. Three QTLs were identified for SB on chromosome 4B, but their map positions and positive allele contribution were inconsistent in the 2 years (ESM Table 2). A major QTL for plant height (*qPH.4B.1*) was identified on chromosome 4B. This QTL was located in the same marker interval "barc20-gwm368" under drought and control conditions and positive allele was contributed by the tall parent C306. Recently, McIntyre et al. (2010) have also identified a QTL for plant height on chromosome 4B with one of the flanking markers (barc20) being common to our results. This QTL corresponds to the *Rht1b*, a known gene for plant height in wheat (Cadalen et al. 1998).

Under control conditions, QTLs were identified for five yield and biomass traits in both the years, with the exception of HI which was significant only in the year 2010 (ESM Table 3). Two QTLs, *qGY.7B.1* and *qGY.2D.1*, explaining 7 and 9 % of the phenotypic variance for grain yield per plant were detected on chromosome 7B and 2D in 2009 and 2010, respectively. The positive alleles for yield were contributed by the high yielding parent WL711. A single QTL was identified for HI in control conditions on chromosome 2D in 2010, positive allele for which was contributed by WL711. Other significant QTLs identified were for shoot biomass, one each on chromosome 3D and 4A, explaining 11 and 7 % of the phenotypic variance, respectively with positive allele originating from both the parents (ESM Table 3). Two QTLs were identified for days to flowering on chromosome 2D, each explaining 11 % of the phenotypic variance. These QTLs

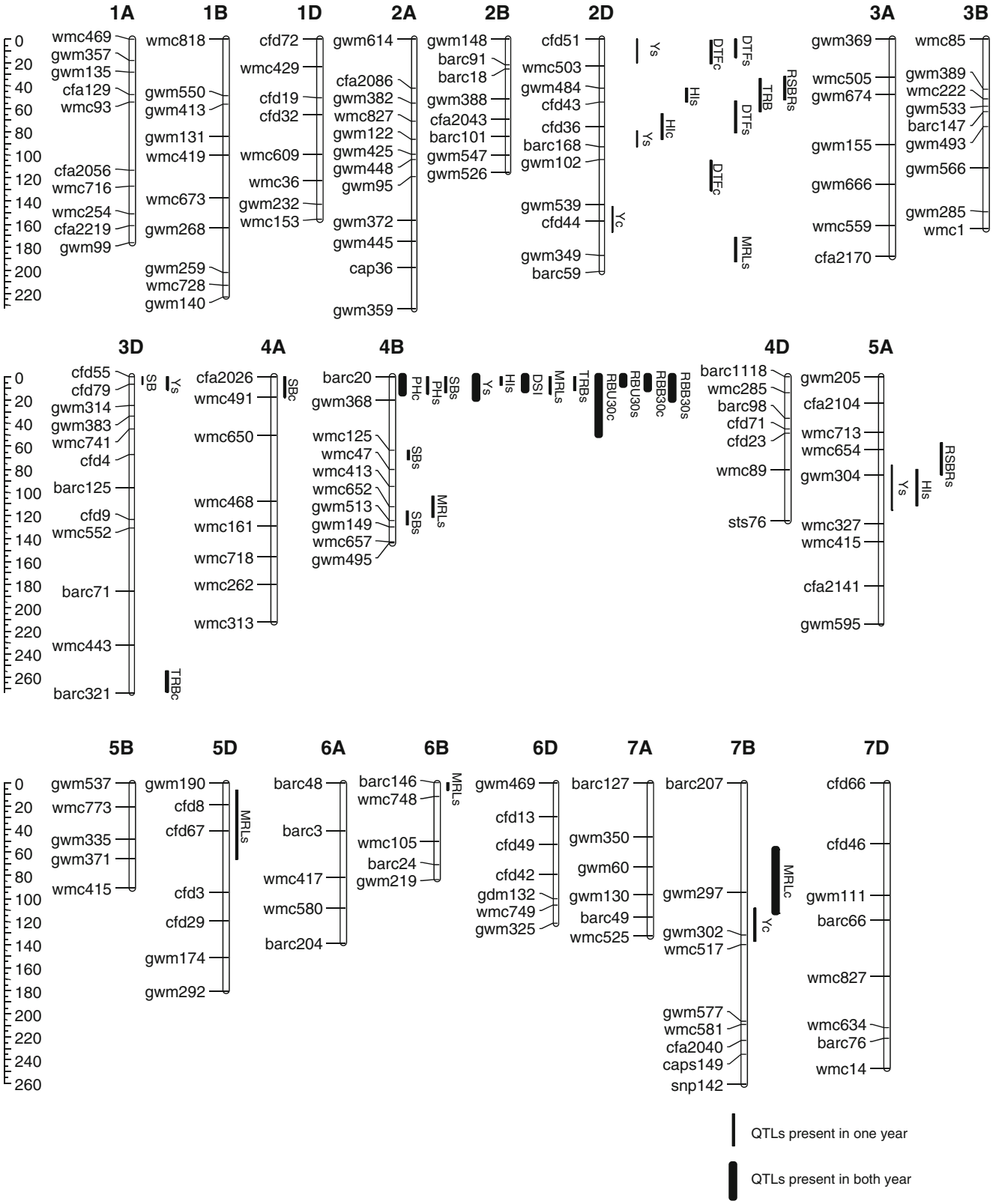


Fig. 3 Molecular genetic map of 21 wheat chromosomes based on WL711/C306 RIL population showing location of QTLs for drought-tolerance traits. The scale on the *left side* of chromosome bars shows map distances in cM (Kosambi 1944). QTL intervals are indicated by *vertical black bars*

Table 4 Main effect QTLs and QTL x environment interactions for different drought-related traits identified by two locus analysis using QTL Network software in 206 WL711/C306 wheat RILs grown in pipes under drought conditions

Traits	QTL	Flanking marker	*a*	ae	h^2(a)%	h^2(ae)%
GY	*qGY.2D.1*	cfd43-cfd36	0.7553***		11.09	
	qGY.3D.1	cfd55-cfd79	−0.3011***	−0.21* (ae2)	8.17	1.65
	qGY.4B.1	barc20-gwm368	−0.0411***		6.69	
	qGY.5A.1	gwm304-wmc327	0.1574***	0.41* (ae1), 0.43* (ae2)	5.31	1.33
DSI	*qDSI.4B.1*	barc20-gwm368	0.1833***		12.34	
HIs	*qHI.2D.1*	cfd36-barc168	3.1498***		14.54	
	qHI.4B.1	barc20-gwm368	−2.0177***		13.11	
	qHI.5A.1	gwm304-wmc327	−0.5442***	−2.45*(ae2)	6.66	0.65
SBs	*qSB.4B.1*	barc20-gwm368	−0.1142***		7.44	
DTFs	*qDTF.2D.1*	cfd43-cfd36	−1.4911***		5.21	
PHs	*qPH.4B.1*	barc20-gwm368	−2.4595***		8.67	
MRLs	*qMRL.4B.1*	barc20-gwm368	−1.0299***	−2.00** (ae1), −2.03** (ae2)	5.02	2.01
TRBs	*qTRB.2D.1*	gwm484-cfd43	−0.5108***		9.45	
	qTRB.4B.1	barc20-gwm368	−0.4373***		10.89	
RBU30	*qRBU30.4B.1*	barc20-gwm368	0.0693***		8.22	
RBB30	*qRBB30.4B.1*	barc20-gwm368	−0.5465***		14.48	

Traits abbreviations are given in Table 1

a additive main effects, *ae1* the additive QTL×environment interaction effects in E1, *ae2* the additive QTL×environment interaction effects in E2; a positive value indicates that the WL711 allele, and a negative value that the C306 allele; h^2 *(a)%* phenotypic variation explained (PVE) by a effects; h^2 *(ae)%*, PVE by ae effects

*P<0.05, **P<0.01, ***P<0.005. Only significant effects are listed

were present at slightly different map positions but both were contributed by the late flowering parent C306. Most of these QTLs were significant in only 1 year and showed small effects (Fig. 3). The *qPH.4B.1* QTL for PH was also identified under control condition in both the years, explaining phenotypic variance of 18 and 22 %. No significant epistatic interaction was observed, suggesting that the identified QTLs have mainly additive gene effects on the respective traits.

Genomic associations with root traits

Fourteen genomic intervals were identified for the four root traits investigated; nine of these were detected under drought conditions and five under control conditions (Tables 4 and 5; ESM Tables 2, 3). Of the nine genomic intervals identified under drought, two (*qRBU30.4B.1* and *qRBB30.4B.*1) were detected in both the years and seven were detected in 1 year only. The positive alleles for seven of the nine root QTLs identified under drought were from drought-tolerant parent C306. For two QTLs, *qMRL.4B.2* and *qRBU30.4B.1*, positive allele came from the drought-sensitive parent WL711, suggesting that alleles from both the parents contributed for a higher root biomass. Individual QTLs for root traits under drought explained 4–27 % of the phenotypic variation. Five genomic intervals were identified for MRL but none of these were consistent in both the years (Table 4; ESM Table 2). A consistent genomic region for root biomass below 30 cm (*qRBB30.4B.1*) was identified on chromosome 4B in both the years. It was co-located with QTLs for maximum root length and total root biomass in 2009 and root biomass up to 30 cm in both the years.

Under control conditions, five root genomic regions were identified of which three were identified in both the years and two in a single year. A consistent QTL for maximum root length under control condition (*qMRL.7B.1*) was identified on chromosome 7B in both the years with positive allele coming from WL711. Two genomic regions for total root biomass were identified in 2009 only and positive alleles for both of these were contributed by C306. A consistent genomic region for RBU30 was contributed by drought-sensitive parent WL711, whereas an alternate genomic region for RBB30 was contributed by the drought-tolerant parent C306 in the same region of chromosome 4B explaining 11 and 9 % of the total phenotypic variance, respectively (Table 4; ESM Table 3). These results provide important clue to resolving the complex genetics of root traits in wheat in relation to drought tolerance.

Epistatic and Meta QTLs

Epistatic QTLs showing QTL×environment (QE), QTL×QTL (QQ), and QTL×QTL×environmental (QQE)

Table 5 Main effect QTLs and QTL×environment interactions for different drought-related traits identified by two locus analysis using QTL Network software in 206 WL711/C306 wheat RILs grown in pipes under control conditions

Traits	QTL	Flanking marker	*a*	ae	h^2(a)%	h^2(ae)%
Yc	qGY.2D.1	gwm539-cfd44	0.51		8.24	
HIc	qHI.2D.1	cfd36-barc168	1.86	−0.78 (ae1), 0.78* (ae2)	14.36	1.39
SBc	qSB.3D.1	cfd55-cfd79	−1.24		10.02	
DTFc	qDTF.2D.1	gwm102-gwm539	−1.76		5.09	
PHc	qPH.4B.1	barc20-gwm368	−4.78		16.76	
MRLc	qMRL.7B.1	barc207-gwm297	4.02		6.29	
TRBc	qTRB.2D.1	gwm484-cfd43	−0.75		6.69	
RBU30c	qRBU30.4B.1	barc20-gwm368	0.40		5.3	
RBB30c	qRBB30.4B.1	barc20-gwm368	−0.49		10.15	

All the other notes are the same as shown in Table 4

interactions were studied to gain a better understanding of the genetic bases of these traits in wheat. The results of main effect QTL and QE interactions for drought and control environments are shown in Tables 4 and 5, respectively. In drought conditions, grain yield QTLs *qGY.3D.1* and *qGY.5A.1* were involved in QE interaction. QE interaction was also identified for HI and MRL QTLs *qHI.5A.1* and *qMRL.4B.1*. Most of the QE interactions was detected in the year 2010 which presented a harsher drought conditions (Table 4). Only one QE interaction was identified for *qHI.2D.1* in both years under control conditions (Table 5). The QTLs with significant QQ and QQE interactions are shown in ESM Table 4. Only one digenic epistatic (QQ) interaction was detected for SB and two QQ interactions were identified for DTF under drought. Shoot biomass QTLs *qSB.2A.1 and qSB.4A.1* also showed significant QQE interaction. Under control conditions, one QQ interaction for DTF and two QQ interactions for PH, but no significant QQE effect were identified (ESM Table 5). Interestingly, the most important genomic region on chromosome 4BS identified in the present study did not show any significant QE, QQ, or QQE interaction.

We also conducted meta-analysis of QTLs to identify consensus genomic associations across environments and to refine QTL positions on the genetic linkage map (Goffinet and Gerber 2000). Meta-analysis was performed on QTL clusters on each chromosome using Biomercator v2.0 (http://www.genoplante.com). The Akaike Information Criterion (AIC) was used to select the QTL model on each chromosome (Hirotugu 1974). According to this, the QTL model with the lowest AIC value is considered a significant model, indicating the number of meta-QTL (MQTL). QTL meta-analysis requires independent QTLs for the same trait obtained from different plant populations, different locations or environmental conditions (Goffinet and Gerber 2000). In this study, a total of 10 MQTLs were identified of which four were located on chromosome 4B, three on chromosome 2D, two on chromosome 3D, and one on chromosome 5A, using QTLs identified in single locus analysis as inputs (ESM Fig. 1, ESM Table 6).

Differentially expressed genes between parents and RIL bulks

We analyzed differential expression of genes under control and drought stress for identification of drought-responsive genes in the tolerant and sensitive genotypes. In the sensitive parent WL711, a total of total 1,814 gene probes showed upregulation and 3,896 probes downregulation, whereas in the tolerant parent C306, 1,297 gene probes showed upregulation and 2,088 probes downregulation of expression in response to drought (Table 6). Out of 3,385 probes differentially expressed in C306, 1,835 were unique and the remaining probes were common to both the parents. Thus, thousands of genes were differentially expressed in response to drought stress in both sensitive and tolerant parents, but only some of these would be responsible for the drought tolerance in C306 or sensitivity in WL711. A more relevant comparison for identifying genes for drought tolerance would be to examine differential expression of genes between the tolerant and sensitive parents. By taking expression levels in WL711 as base, we found 962 probes overexpressed and 2,251 probes underexpressed in C306 under control conditions. Under drought stress, the number of up- and downregulated gene probes was 818 and 1,315, respectively (Table 6).

Most of the differentially expressed genes between WL711 and C306 would not be responsible for the difference in their drought tolerance, as many of these could be related to other genetic differences between the two diverse cultivars. Therefore, we analyzed the profiles of bulked RNA from 10 each of the most tolerant and sensitive RILs to normalize the background noise of differentially expressed genes not related to drought tolerance (Pandit et

Table 6 Number of differentially expressed gene probes at $P<0.01$ and log fold change of >2.0 between drought tolerant and drought sensitive genotypes under control and drought stress conditions

Genotypes/Genes	Control condition			Drought condition		
	Up	Down	Total	Up	Down	Total
WL711	–	–	–	1,814	3,896	5,710
C306	–	–	–	1,297	2,088	3,385
Sensitive RIL bulk	–	–	–	2,467	2,500	4,967
Tolerant RIL bulk	–	–	–	1,764	1,361	3,125
WL711 versus C306[a]	962	2,251	3,213	818	1,315	2,133
Sensitive versus tolerant RIL bulk[b]	774	754	1,528	574	671	1,243
Common in tolerant parent/tolerant bulk	–	–	–	76	86	162
Genes in the *qDSI.4B.1* region on short arm of 4B	–	–	–	1	4	5

[a] Taking signals from the sensitive parent WL711 as base

[b] Taking signals from the sensitive RIL bulk as base

al. 2010). Under control conditions, 774 gene probes showed upregulation and 754 probes downregulation of expression in the tolerant bulk as compared to sensitive bulk. The corresponding number of up- and downregulated genes under drought stress was 574 and 671, respectively (Table 6). Thus, there were 1,243 gene probes differentially expressed between the tolerant and sensitive RIL bulks under drought as compared to 2,133 probes between the two parents (Table 6). We further identified 162 gene probes which were commonly differentially expressed between the two parents as well as the two RIL bulks. These are more likely candidates for the differential drought tolerance between C306 and WL711 (Table 6, ESM Table 7). Twenty-four of these genes are of particular interest as their expression was changed more than fivefold, 18 of which are of unknown function. Fifteen of these genes were upregulated and nine were downregulated in the tolerant genotypes as compared to sensitive genotypes under drought.

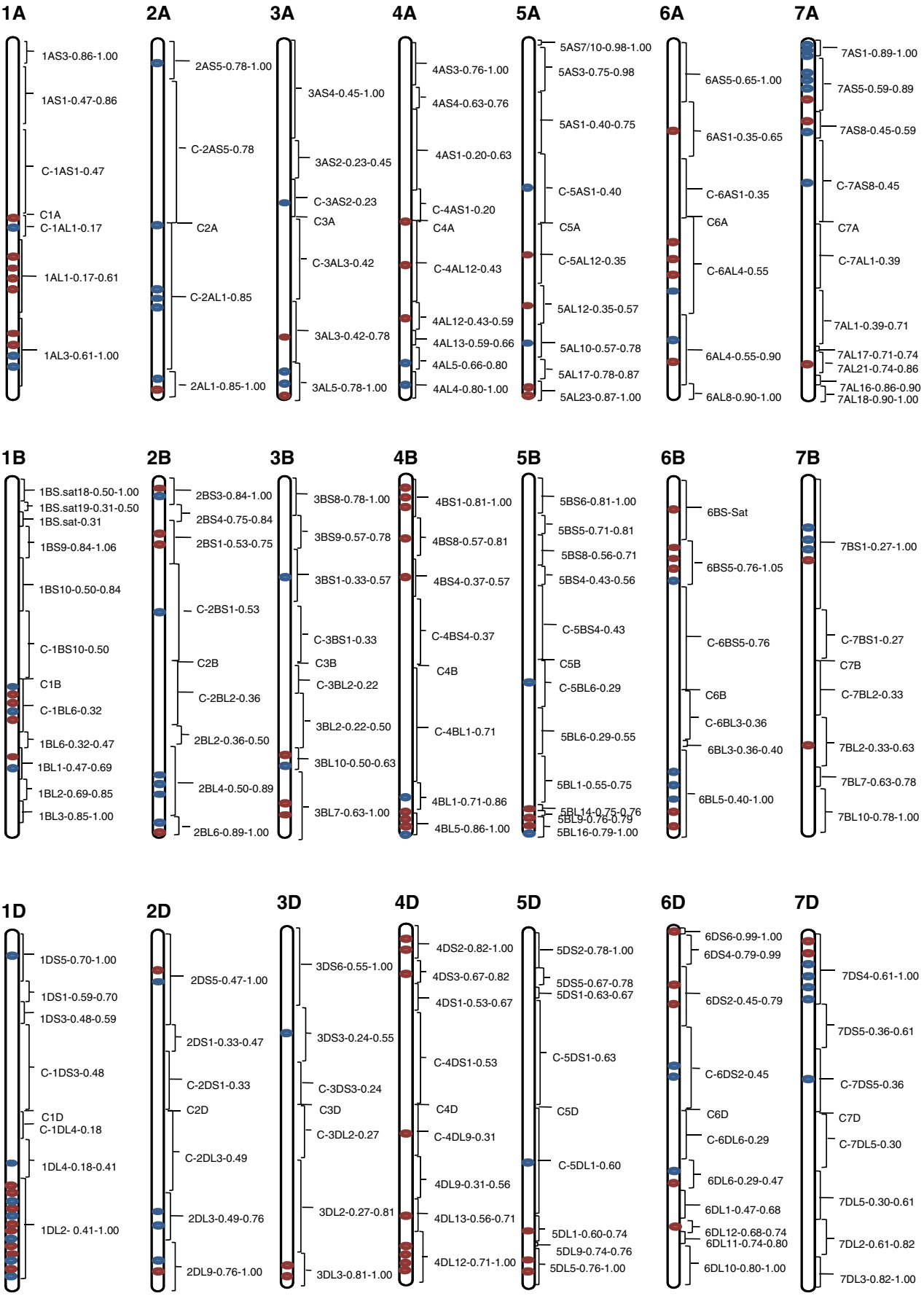
Among the genes with highest modulation of expression level, a gene of unknown function (Affymetrix probe Id. Ta12896.1) was upregulated by 98.24-fold in the tolerant bulk as compared to sensitive bulk (ESM Table 7). Another gene with unknown function (Affymetrix probe Id. Ta28761.1) was upregulated 33.58-fold. Similarly, a gene for sucrose fructan 6-fructosyl transferase was downregulated 25.99-fold and two other genes showing homology with unknown proteins of *Hordeum vulgare* (Ta.8108.2.S1_at Ta.8108.2.S1_x_at) showed downregulation of 26.70- and 16.47-fold, respectively (ESM 7). Nucleotide sequence of the 162 differentially expressed probes were BLASTN searched in a database of bin-mapped wheat ESTs (www.wheat.pw.usda.gov/wEST/blast/). Only 55 probes showed significant hits, nearly two-thirds of the gene probes are not yet bin-mapped in wheat. Map positions of the 55 bin-mapped probes were clustered on the long arm of homoeologous group 1 and short arm of homoeologous group 7 chromosomes (Fig. 4). In addition, there was significant concentration of differentially expressed genes in the bins of chromosome 4B, 4D, 6AL, and 6BL.

Differentially expressed genes in the qDSI.4B.1 genomic region

We were particularly interested in the differentially expressed genes in the *qDSI.4B.1* region flanked by SSR markers barc20 and gwm368 on chromosome 4B. This region was spread over three deletion bins of wheat chromosome arm 4BS (www.wheat.pw.usda.gov). On the basis of bin-map information, we identified five differentially expressed gene probes in the *qDSI.4B.1* region (Tables 6 and 7). A random distribution of the 55 bin-mapped probes in the entire wheat genome of about 17,000 Mbp (Gill et al. 2004) predicts one gene per 309 Mbp, or 67.6 cM of the total 3,720 cM map distance estimated in the present study. Location of five differentially expressed genes in the 12 cM interval for *qDSI.4B.1* shows 28.1-fold enrichment of differentially expressed genes in this genomic region. These five genes are likely candidates for drought tolerance in this genomic region (Table 7). However, nearly two-thirds (107 genes) of the 162 differentially expressed genes are not yet bin-mapped in wheat and some of these are also likely to be located in *qDSI.4B.1* genomic region.

Synteny of qDSI.4B.1 genomic region in other cereal species

The *qDSI.4B.1* genomic region of wheat chromosome 4B shows synteny with rice chromosome 3 (Sorrells et al. 2003; Singh et al. 2007). QTLs for root traits have already been mapped in the syntenic region of rice chromosome 3 (Yue et


1A
1AS3-0.86-1.00
1AS1-0.47-0.86
C-1AS1-0.47
C1A
C-1AL1-0.17
1AL1-0.17-0.61
1AL3-0.61-1.00
2A
2AS5-0.78-1.00
C-2AS5-0.78
C2A
C-2AL1-0.85
2AL1-0.85-1.00
3A
3AS4-0.45-1.00
3AS2-0.23-0.45
C-3AS2-0.23
C3A
C-3AL3-0.42
3AL3-0.42-0.78
3AL5-0.78-1.00
4A
4AS3-0.76-1.00
4AS4-0.63-0.76
4AS1-0.20-0.63
C-4AS1-0.20
C4A
C-4AL12-0.43
4AL12-0.43-0.59
4AL13-0.59-0.66
4AL5-0.66-0.80
4AL4-0.80-1.00
5A
5AS7/10-0.98-1.00
5AS3-0.75-0.98
5AS1-0.40-0.75
C-5AS1-0.40
C5A
C-5AL12-0.35
5AL12-0.35-0.57
5AL10-0.57-0.78
5AL17-0.78-0.87
5AL23-0.87-1.00
6A
6AS5-0.65-1.00
6AS1-0.35-0.65
C-6AS1-0.35
C6A
C-6AL4-0.55
6AL4-0.55-0.90
6AL8-0.90-1.00
7A
7AS1-0.89-1.00
7AS5-0.59-0.89
7AS8-0.45-0.59
C-7AS8-0.45
C7A
C-7AL1-0.39
7AL1-0.39-0.71
7AL17-0.71-0.74
7AL21-0.74-0.86
7AL16-0.86-0.90
7AL18-0.90-1.00
1B
1BS.sat18-0.50-1.00
1BS.sat19-0.31-0.50
1BS.sat-0.31
1BS9-0.84-1.06
1BS10-0.50-0.84
C-1BS10-0.50
C1B
C-1BL6-0.32
1BL6-0.32-0.47
1BL1-0.47-0.69
1BL2-0.69-0.85
1BL3-0.85-1.00
2B
2BS3-0.84-1.00
2BS4-0.75-0.84
2BS1-0.53-0.75
C-2BS1-0.53
C2B
C-2BL2-0.36
2BL2-0.36-0.50
2BL4-0.50-0.89
2BL6-0.89-1.00
3B
3BS8-0.78-1.00
3BS9-0.57-0.78
3BS1-0.33-0.57
C-3BS1-0.33
C3B
C-3BL2-0.22
3BL2-0.22-0.50
3BL10-0.50-0.63
3BL7-0.63-1.00
4B
4BS1-0.81-1.00
4BS8-0.57-0.81
4BS4-0.37-0.57
C-4BS4-0.37
C4B
C-4BL1-0.71
4BL1-0.71-0.86
4BL5-0.86-1.00
5B
5BS6-0.81-1.00
5BS5-0.71-0.81
5BS8-0.56-0.71
5BS4-0.43-0.56
C-5BS4-0.43
C5B
C-5BL6-0.29
5BL6-0.29-0.55
5BL1-0.55-0.75
5BL14-0.75-0.76
5BL9-0.76-0.79
5BL16-0.79-1.00
6B
6BS-Sat
6BS5-0.76-1.05
C-6BS5-0.76
C6B
C-6BL3-0.36
6BL3-0.36-0.40
6BL5-0.40-1.00
7B
7BS1-0.27-1.00
C-7BS1-0.27
C7B
C-7BL2-0.33
7BL2-0.33-0.63
7BL7-0.63-0.78
7BL10-0.78-1.00
1D
1DS5-0.70-1.00
1DS1-0.59-0.70
1DS3-0.48-0.59
C-1DS3-0.48
C1D
C-1DL4-0.18
1DL4-0.18-0.41
1DL2- 0.41-1.00
2D
2DS5-0.47-1.00
2DS1-0.33-0.47
C-2DS1-0.33
C2D
C-2DL3-0.49
2DL3-0.49-0.76
2DL9-0.76-1.00
3D
3DS6-0.55-1.00
3DS3-0.24-0.55
C-3DS3-0.24
C3D
C-3DL2-0.27
3DL2-0.27-0.81
3DL3-0.81-1.00
4D
4DS2-0.82-1.00
4DS3-0.67-0.82
4DS1-0.53-0.67
C-4DS1-0.53
C4D
C-4DL9-0.31
4DL9-0.31-0.56
4DL13-0.56-0.71
4DL12-0.71-1.00
5D
5DS2-0.78-1.00
5DS5-0.67-0.78
5DS1-0.63-0.67
C-5DS1-0.63
C5D
C-5DL1-0.60
5DL1-0.60-0.74
5DL9-0.74-0.76
5DL5-0.76-1.00
6D
6DS6-0.99-1.00
6DS4-0.79-0.99
6DS2-0.45-0.79
C-6DS2-0.45
C6D
C-6DL6-0.29
6DL6-0.29-0.47
6DL1-0.47-0.68
6DL12-0.68-0.74
6DL11-0.74-0.80
6DL10-0.80-1.00
7D
7DS4-0.61-1.00
7DS5-0.36-0.61
C-7DS5-0.36
C7D
C-7DL5-0.30
7DL5-0.30-0.61
7DL2-0.61-0.82
7DL3-0.82-1.00

◀ **Fig. 4** Distribution of 55 differentially expressed genes in wheat chromosome bins. *Red dots* show downregulation and *blue dots* upregulation of expression in tolerant RILs as compared to sensitive RILs

al. 2006; Qu et al. 2008). Five differentially expressed genes identified in the *qDSI.4B.1* region were spread over three bins on the short arm of wheat chromosome 4B. Orthologs of one of these genes, coding for thiamine biosynthesis protein were identified in the syntenic region of each of the genomes of rice, sorghum, and maize, whereas orthologs of another gene, a serine threonine protein kinase was present on rice chromosome 3 only (Fig. 5, ESM Table 8). In addition, BLASTN search was performed with the 107 unmapped differentially expressed wheat gene probes against genomic sequences of rice chromosome 3 (http://rgp.dna.affrc.go.jp/IRGSP/download.html), sorghum chromosome 1 (www.phytozome.net), and maize chromosome 1 (www.maizesequence.org). Six of these genes, including a heat shock protein, a histone-like protein, a zinc finger protein, a Wiscott–Aldrich C-terminal protein, and two unknown proteins showed homology in the syntenic 26–32 Mb region of rice chromosome 3 (Fig. 5; ESM Table 8). The wheat *qDSI.4B.1* region is syntenic to short arm of sorghum chromosome 1. In addition to the location of conserved gene for thiamine biosynthesis protein, there were three other differentially expressed wheat genes that were common between rice and sorghum, namely a heat shock protein, a histone-like protein and a homolog of unknown predicted protein from *Hordeum vulagre* (ESM Table 8). This genomic region of sorghum chromosome 1 has homologs of two other differentially expressed wheat genes, a 3-ketoacetyl-thiolase and an unknown protein. Drought tolerance related stay green trait has also been mapped in this region of sorghum chromosome 1 (Fig. 5). The wheat *qDSI.4B.1* region is syntenic to the long arm of maize chromosome 1 and a QTL affecting anthesis-silking interval under drought has been mapped in this region of maize chromosome 1 (Messmer et al. 2009). Apart from the conserved thiamine biosynthesis gene, this region of maize chromosome 1 has other homologs of differentially expressed wheat genes which are not yet bin-mapped in wheat, namely histone protein, Rubisco large subunit, ribosomal protein S7, and ATP synthase cf1 beta subunit. Homologs of three of the five genes differentially expressed between the two parents and RIL bulks were unique to wheat and did not map in the syntenic genomic regions of rice, maize, or sorghum.

Discussion

Development of drought-tolerant wheat varieties is high priority due to unpredictable rainfall patterns and impending climate change (Tester and Langridge 2010; Fleury et al. 2010). Here, we analyzed 10 different shoot and root traits under drought and control conditions for the identification of genomic regions and candidate genes associated with drought tolerance.

Consistent genomic associations with drought-tolerance traits

A number of traits have been associated with genetic variation for drought tolerance in wheat, but only few genomic regions have been genetically mapped, fewer still have been utilized in breeding and none have been cloned (Richards 2006; Olivares-Villegas et al. 2007; Collins et al. 2008; Reynolds and Tuberosa 2008; Fleury et al. 2010). In the present study, SB, HI, DTF, MRL, TRB, and GY under drought stress were controlled by multiple genomic regions, highlighting the complex nature of drought adaptation traits, but consistent genomic associations were located only on chromosomes 4B and 7B (Fig. 3). Genomic region for DSI was co-located with those for GY, HI, and TRB on the chromosome arm 4BS. Co-location of genomic regions for multiple traits is indicative of either pleiotropic effect of a single gene or cluster of tightly linked genes affecting different traits (Huang et al. 2004). Earlier, Quarrie et al. (2005) mapped strongest QTLs for grain yield under drought on chromosome arms 7AL and 7BL, but they did find significant genomic associations on chromosome arm 4BS near the *Rht1b* gene for plant height. Interestingly, a

Table 7 Annotation of genes within the genomic region *qDSI.4B.1* that were commonly differentially expressed between the two RIL bulks as well as the two parental wheat lines C306 and WL711

Sr. no.	Affymetrix Probe Set Id	BLASTX Accession Id	Wheat EST	Fold change	Putative function
1	Ta.28078.1.A1_s_at	XP_002880287.1	BE591450	−2.31	Myb family transcription factor
2	Ta.5331.1.A1_a_at	AAX19515.1	BF428648	−2.56	Serine/threonine protein kinase
3	Ta.22324.1.S1_at	BAK02456.1	BE604060	−2.24	Unknown barley protein
4	Ta.22443.1.S1_at	AAK26130.1	CD453979	−2.41	Thiamine biosynthesis protein
5	Ta.3052.1.S1_at	NP_001105512.1	CD373618	+2.77	Diphosphonucleotide phosphatase 1

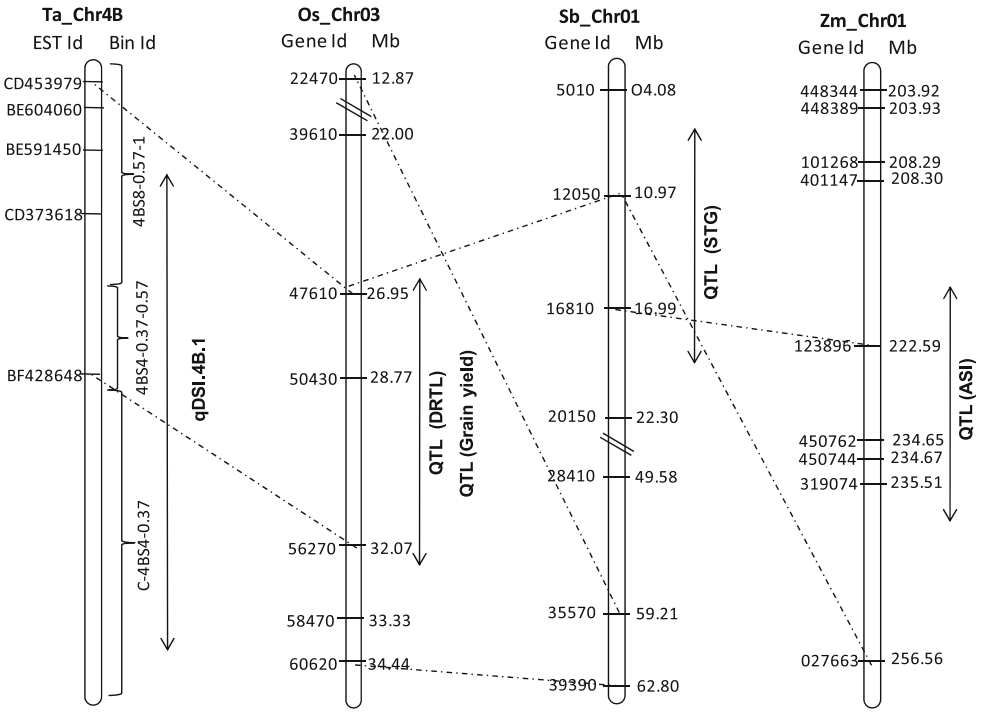


Fig. 5 Colinearity of genes and QTLs in the *qDSI4B.1* region of wheat chromosome 4B with syntenic regions of rice, maize, and sorghum. *Dotted lines* connect positions of orthologous genes. *Arrows* indicate positions of QTLs. Only last digits of the respective gene Id are shown. Full gene Id include prefix of LOC_Os03g for rice (http://rgp.dna.affrc.go.jp), Sb01g for sorghum (www.phytozome.net), GRMZM2G for maize (www.maizeseqence.org)

consistent genomic region for grain yield under drought (*qDTY1.1*) has been mapped on rice chromosome 1 that is also tightly linked to plant height gene (*Sd1*) in a tall drought-tolerant landrace of rice "N22" (Vikram et al. 2011). Close linkage between plant height genes and yield under drought needs detailed investigation by fine mapping and loss of function mutagenesis in both these instances.

The *qDSI4B.1* appears to be the most important genomic region for drought tolerance in wheat variety "C306". Earlier studies have also reported QTLs for drought tolerance in the same region of chromosome 4B (McCartney et al. 2005; Marza et al. 2006; Yang et al. 2007; Dashti et al. 2007; Diab et al. 2008; Rebetzke et al. 2008; Mathews et al. 2008; McIntyre et al. 2010; Pinto et al. 2010). Dashti et al. (2007) identified QTL for stress susceptibility index on chromosome 4B linked to *Rht1b* gene. However, Pinto et al. (2010) did not find linkage with any known *Rht* genes in the Seri/Babax mapping population, suggesting that linkage between drought tolerance and plant height in the WL711/C306 population may be coincidental. The probability of identifying genes for minor effect is enhanced by avoiding segregation of genes for major phenological traits as demonstrated by subdividing Kukri/RAC875 population into early and late subpopulations for QTL analysis (Reynolds and Tuberosa 2008; Reynolds et al. 2009). There was a relatively narrow range of phenology in the present WL711/C306 population, except for plant height. Contrary to earlier reports (McCartney et al. 2005), we found no consistent genomic associations for yield under stress with days to flowering. Genomic regions for grain yield, thousand grain weight, phenology, water-soluble carbohydrates, grain number, canopy temperature, and carbon isotope discrimination under drought have also been mapped on wheat chromosomes other than 4B (Kuchel et al. 2007; Yang et al. 2007; Rebetzke et al. 2008; Olivares-Villegas et al. 2008; Diab et al. 2008; Maccaferri et al. 2008). Meta-analysis of QTLs for grain yield and related traits has identified 55 genomic regions on chromosomes 1A, 1B, 2A, 2D, 3B, 4A, 4B, 4D, and 5A (Zhang et al. 2010b).

In comparison to other cereals, little attention has been paid to genetic analysis of root traits in wheat under reproductive stage drought (Fleury et al. 2010). Genomic regions have been identified for root volume and grain yield under drought on rice chromosome 3, in a region syntenic to wheat chromosome 4B (Venuprasad et al. 2002; Yue et al. 2006; Qu et al. 2008; Swamy et al. 2011). In barley, chromosome 4H is reported to carry important genes for adaptation to water stress (Handley et al. 1994; Bezant et al. 1997). Similar correspondence between grain yield under drought has been reported in syntenic genomic regions of maize and sorghum chromosome 1 (Messmer et al. 2009). Functional and structural relatedness of the syntenic region of *qDSI.4B.1* will help cross-validate the candidate genes in other cereals and transfer information from one crop species to another for gene discovery. The region of chromosome 4B is especially interesting due to co-localization of several QTLs for shoot and root biomass with trait-enhancing allele coming from the drought-tolerant parent C306. Deeper roots, especially the seminal roots are considered important for wheat growth under drought (Sanguineti et al. 2007;

Araus et al. 2008). Recently, Ren et al. (2011) have reported QTLs for lateral root length, total root length, and root tip number for seminal roots at seedling stage on wheat chromosome 4B with a common flanking marker gwm368, mapped in the present study. This shows that wheat chromosome arm 4BS is a hot spot of genes for important root traits and plays a key role in the drought tolerance of wheat variety C306. Fine mapping of this region will be useful for the identification of genes for drought tolerance and breeding applications in wheat.

Candidate genes for drought tolerance in the associated genomic regions

The idea behind transcriptome profiling of bulked extreme RILs was to normalize the random differential expression of genes between the tolerant and sensitive parents, while retaining the differential expression of genes relevant to drought tolerance (Pandit et al. 2010). There were 1,243 gene probes differentially expressed between the tolerant and sensitive RIL bulks under drought, which was still quite a large number, therefore we focused on 162 genes which were commonly differentially expressed between the two parents and two RIL bulks. These are more likely candidates for the drought tolerance in C306.

There are reports of direct correlation between proline accumulation and ability of plants to tolerate abiotic stresses (Kishor et al. 2005; Seki et al. 2007). However, eightfold downregulation of proline-rich protein (Ta.21419.2.S1_at) in our study supports recent reports of lower accumulation of proline in tolerant genotypes at early stages of salt and drought stress in wheat (Poustini et al. 2007; Xue et al. 2008; Ergen et al. 2009). Similarly, glutathione transferase (Ta.303.1.S1_at) was downregulated in the tolerant RILs under drought stress. Glutathione is an important antioxidant, redox buffer, and detoxifier (Noctor and Foyer 1998; Mittler et al. 2004; Ergen et al. 2009); therefore, it was surprising to see downregulation of glutathione transferase in our study. Mohammadi et al. (2007) have also observed downregulation of glutathione-related genes under water stress.

Expression of genes involved in polysaccharide metabolism, especially cell wall polymers, is modulated by osmotic stress (Sahi et al. 2006). We identified stress-responsive hydrolases and transferases that putatively act on glucans. There was increase in the expression of beta-glucanases (Ta.10.1.S1_a_at) that promote loosening and remodeling of cell wall polysaccharides. Cell wall loosening facilitates growth and decreases water potential of cells to compensate for the decrease in water potential gradient under drought stress (Hincha et al. 1997; Cho et al. 2006; Mohammadi et al. 2007). We observed upregulation of specific transporters of water, sugars, and peptides. A *nod26*-like major intrinsic protein was the only aquaporin detected among the drought-responsive transcripts. Putative transporters of sucrose/fructan 6-fructosyl transferase was regulated, which reflects attempts to restore ionic and osmotic balance. *OPT* classes of oligopeptide transporters are also known to be induced by drought (Waterworth and Bray 2006; Mohammadi et al. 2007). Transcription factor genes regulated by drought stress, such as zinc finger and MYB genes are also regulated by heat stress (Barnabas et al. 2008; Qin et al. 2008). We found that a gene involved in GA biosynthesis, *2OG-Fe oxygenase*, was downregulated under drought stress but role of GA in abiotic stress tolerance is debatable (Vettakkorumakankav et al. 1999). Induction of cytochrome *P450* genes is consistent with their protective role under biotic and abiotic stresses (Guo et al. 2007). A large number of drought-regulated transcripts were of unknown function, showing large gap in our knowledge on the identity of genes involved in the complex drought response pathway.

QTL mapping studies have identified numerous genomic regions associated with abiotic stress tolerance in crop plants. But, only in few instances, the functional alleles of genes underlying the QTL have been identified. Wayne and McIntyre (2002) were among the first to demonstrate the value of combining genetic mapping with genome wide expression profiling to narrow down the candidate genes for a complex trait, viz. ovariole number in *Drosophila*. Integration of linkage mapping and expression profiling has been used in plants to identify genes underlying complex agronomic trait in maize (Marino et al. 2009) and rice (Pandit et al. 2010; Deshmukh et al. 2010). However, no such studies have been reported in wheat and could be prohibitively expensive when applied to large number of samples. We reduced the number of samples for transcriptome profiling by bulking the RILs with extreme DSI phenotypes. We were particularly interested in differentially expressed genes in the *qDSI.4B.1* region flanked by SSR markers barc20 and gwm368 on wheat chromosome 4B. Among the five differentially expressed genes in this region, a serine/threonine protein kinase homolog (AAX19515.1, *Triticum aestivam*) was constitutively downregulated in C306 and tolerant RILs. Earlier, it has been shown that wheat *TaSnRK2.4*, an *SNF1*-type serine/threonine protein kinase enhanced multistress tolerance in *Arabidopsis* (Mao et al. 2010; Zhang et al. 2010a). Another differentially expressed gene in this genomic region was a *Myb* transcription factor (XP_002880287.1 *Arabidopsis lyrata*) which was downregulated in the tolerant parent and RILs. Cai et al. (2011) have identified the role of *MYB3R* gene in drought, salt, and cold stress tolerance in wheat. Expression of a homolog of predicted barley protein (BAK02456.1 *H. vulgare*) and a putative thiamine biosynthesis protein (AAK26130.1 *Oryza sativa*), was also downregulated in the tolerant parent and RILs under drought. Expression of

only one gene coding for diphosphonucleotide phosphatase 1 (NP_001105512.1 *Zea mays*) was upregulated. Recently, Ji et al. (2011) have shown that drought-tolerant wheat varieties accumulate lower levels of ABA at the terminal stage drought in comparison to sensitive varieties. These results and recently published QTL mapping studies (Mathews et al. 2008; McIntyre et al. 2010) underline the importance of *qDSI.4B.1* region for drought tolerance in wheat. However, confirmation of the role of these genes in drought tolerance will need validation through genetic transformation and association studies.

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